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NOTE

# Differential Role for *BiP3* in Rice Immune Receptor-Mediated Resistance

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**Abstract** Endoplasmic reticulum-bound chaperone luminal-binding protein 3 (*BiP3*) has been found to regulate the immunity mediated by the membrane-bound extracellular immune receptors *Xa3/Xa26* and *Xa21*, that encode non-arginine-aspartate (non-RD) kinases, against the bacterial pathogen *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*). In contrast, *BiP3* appeared not to regulate the immunity mediated by the intracellular immune receptor *Pi5*, which encodes a nucleotide-binding domain and leucine-rich repeat (NB-LRR) protein, against the fungal pathogen *Magnaporthe oryzae*. To further examine this differential role for *BiP3* in rice immunity, we generated transgenic rice plants overexpressing *BiP3* in the background of the NB-LRR intracellular immune receptor *Xa1* that confers resistance to *Xoo*. Our molecular genetic and phenotype analyses revealed that *BiP3* overexpression does not affect *Xa1*-mediated rice resistance to *Xoo*. Our current results thus provide evidence that *BiP3* regulates membrane-bound non-RD kinase-mediated, but not the intracellular NB-LRR-mediated, rice immune responses and that its function does not depend on the type of pathogen.

**Keywords** endoplasmic reticulum-bound chaperone luminal-binding protein 3 · nucleotide-binding domain and leucine-rich repeat · *Xa1* · *Xanthomonas oryzae* pathovar *oryzae*

## Introduction

Plants sense a wide range of microbes via immune receptors that monitor extracellular or intracellular spaces for microbe-associated molecules (Jones and Dangl, 2006; Boller and Felix, 2009). Based on the currently available data, the cell surface recognition of pathogen-specific molecules is mediated by membrane-bound immune receptors such as Xa3/Xa26 and Xa21, which are a non-arginine-aspartate (non-RD) class of kinases (Song et al., 1995; Sun et al., 2004; Dardick and Ronald, 2006). Plants also contain intracellular immune receptors, which are mostly nucleotide binding-leucine rich repeat (NB-LRR) proteins. The NB-LRR proteins are characterized by a tripartite domain architecture consisting of an N-terminal coiled-coil or Toll/interleukin-1 receptor domain, a central NB domain, and a C-terminal LRR domain (Martin et al., 2003; Liu et al., 2007).

Membrane-bound immune receptors are synthesized in the endoplasmic reticulum (ER), where they are subjected to a quality control process responsible for monitoring the correct folding and processing of membrane and secretory proteins (Li et al., 2009; Nekrasov et al., 2009; Park et al., 2010; Saijo, 2010). Recently the ER-bound HSP70 luminal-binding protein (BiP) was found to be one of the main chaperones regulating the biogenesis and degradation of membrane-bound immune receptors. For example, the *Xa21*-mediated immune response is compromised in *BiP3*-overexpressing rice plants due to a reduced stability of Xa21 upon *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*) inoculation (Park et al., 2010). *BiP3* overexpression also compromises the resistance mediated by rice *Xa3/Xa26* upon *Xoo* inoculation (Park et al., 2014).

In contrast, *BiP3* overexpression does not affect *Pi5*-mediated resistance in rice to the fungal pathogen *Magnaporthe oryzae*, indicating that overexpressed *BiP3* does not have a role in the biogenesis of Pi5 itself or in Pi5-mediated signaling (Park et al., 2014). However, many *Arabidopsis* NB-LRR proteins are found to localize at the ER, suggesting that they may require ER chaperones for maturation (Caplan et al., 2009; Padmanabhan and Dinesh-Kumar, 2010). These previous findings prompted us to examine if the differential effects of overexpressed *BiP3* on the rice immune responses to *Xoo* mediated by the non-RD kinases Xa3/Xa26 and Xa21 and to *M. oryzae* mediated by the *Pi5* encoded NB-LRR are due to different classes of immune receptor

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or different species of pathogen. To address this question, in the present study we generated and analyzed transgenic rice plants overexpressing *BiP3* in a background containing the NB-LRR immune receptor *Xa1* that confers resistance to *Xoo*.

## Materials and Methods

**Plant materials and growth conditions.** The rice cultivar (cv.) Kitaake and monogenic resistance line IRBB1 carrying *Xa1* (Yoshimura et al., 1998) were used as susceptible and resistance control lines, respectively. All rice plants were grown in a greenhouse at 30°C during the day and at 20°C at night with a light/dark cycle of 14/10 h for inoculation and seed harvesting.

**Production of transgenic rice lines.** We generated *BiP3*-overexpressing lines in an IRBB1 background by introducing the BiP3-OX vector carrying maize *Ubiquitin* promoter:*BiP3* fusion using *Agrobacterium*-mediated transformation (Park et al., 2010).

**DNA extraction and genotypic analysis.** Independent transgenic plants were verified by genomic DNA polymerase chain reaction (PCR) analysis. Total genomic DNAs were extracted from the young leaves of seedlings following the method of Chen and Ronald (1999). PCR amplification was performed in a final volume of 40 μL (100 pmol of each primer, 20 μM dNTPs, 10 mM Tris-HCl pH 9.0, 2 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, and 0.5 U Taq polymerase) using 50 ng of genomic DNA as template. The amplification conditions were as follows: 94°C for 5 min followed by 35 cycles of 94°C, 1 min; 56°C, 1 min; and 72°C, 1 min, with a final extension at 72°C for 5 min. The PCR primers used were as follows: *BiP3*, 5'-GCTGCTGCTATTGCGT ACGGTTTGGACA-3' and 5'-AATCATCGCAAGACCGGCAA CAGG-3'; *Xa1*, 5'-ACTGCCCTCTTGCACACGCCTTTGG-3' and 5'-CTGCCAACTGAATTACCAGTTGCA-3'.

**RNA preparation and reverse transcription polymerase chain reaction (RT-PCR) analysis.** To examine the expression of *BiP3* in the leaves of *BiP3-OX* lines, total RNA was prepared using Trizol reagent (Invitrogen, USA) with DNase treatment (TURBO DNA-free kit; Ambion-Life technologies, USA). First-strand cDNAs were amplified with RT-PCR reactions using *BiP3*-specific primers. *OsUBQ5* was amplified as an RT-PCR control using the primers, 5'-GACTACAACATCCAGAAGGAGTC-3' and 5'-TC ATCTAATAACCAGTTCGATTTC-3'. PCR amplifications were performed as described by Han et al. (2013).

**Pathogen inoculation and disease evaluation.** The *Xoo* strain KXO85 (Korean race 1), a causal agent of bacterial blight in rice, was used. KXO85 is incompatible with the IRBB1 background. KXO85 was grown on Peptone Sucrose Agar (PSA; peptone 10, sucrose 10, agar 16, glutamate 1 g/L, pH 7.5) plates with cephalexin (15 mg/L) at 28°C. Fully expanded flag-leaves of each tiller from 10-week-old plants were inoculated with a *Xoo* suspension ($OD_{600}$=0.8) using the scissors-dip method as described by Han et al. (2013). Lesion lengths were measured from the cut surface at the tip to the distal-most position on the leaf that exhibited a grey chlorotic lesion at different intervals after pathogen inoculation. To determine the size of the bacterial populations, three inoculated leaves from each genotype were ground and resuspended in 10 mL $H_2O$ to separately harvest bacteria. Diluted extracts were then plated on PSA media containing 15 mg/L cephalexin.

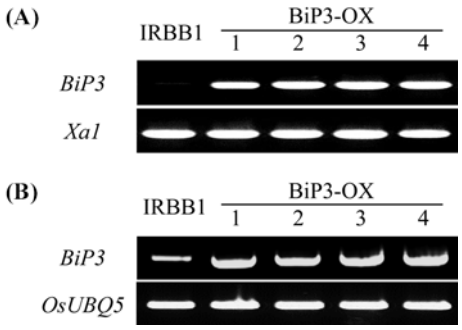


**Fig. 1** Molecular characterization of IRBB1 rice plants carrying *Xa1* and *BiP3*-overexpressing transgenes (BiP3-OX transgenic lines). (A) Genomic DNA PCR analysis of *Xa1* and *BiP3* genes from the IRBB1 and $T_0$ transgenic BiP3-OX lines. (B) RT-PCR analysis of *BiP3*. Control RT-PCR was carried out using *OsUBQ5* specific primers.

## Results and Discussion

Four transgenic rice plants overexpressing *BiP3* in a background containing the NB-LRR immune receptor *Xa1* were generated and the presence of the BiP3-OX transgene in each plant was examined by genomic DNA PCR (Fig. 1A). The expression levels of *BiP3* in the leaves of transgenic plants were monitored by RT-PCR. *BiP3* expression was found to be highly increased in four independent BiP3-OX lines compared to the control IRBB1 line (Fig. 1B). No morphological changes were observed in these transgenic plants compared to the control plants.

To verify whether overexpressed *BiP3* affects *Xa1*-mediated immunity to *Xoo*, two BiP3-OX lines (BiP3-OX1 and BiP3-OX2) were challenged with this pathogen at 10 weeks of age. The IRBB1 line exhibited high resistance to *Xoo* and showed very short lesions of approximately 0.3–0.5 cm following exposure to the incompatible *Xoo* strain KXO85. Transgenic plants overexpressing *BiP3* displayed resistance to *Xoo* with short lesions similar to the IRBB1 control, demonstrating that *BiP3* overexpression does not have an effect on *Xa1*-mediated resistance. Kitaake is a *Xoo* susceptible rice cultivar (Yoshimura et al., 1998) and in our current experiment exhibited long lesions of approximately 15–17 cm (Figs. 2A and B). The bacterial population measurements also correlated well with lesion length development i.e. a much smaller *Xoo* population was detected in BiP3-OX and IRBB1 plants compared with the susceptible Kitaake control plants (Fig. 2C). These results indicated that overexpression of *BiP3* does not compromise *Xa1*-mediated resistance to *Xoo* in rice.

It is now hypothesized that BiP3 is required for the proper

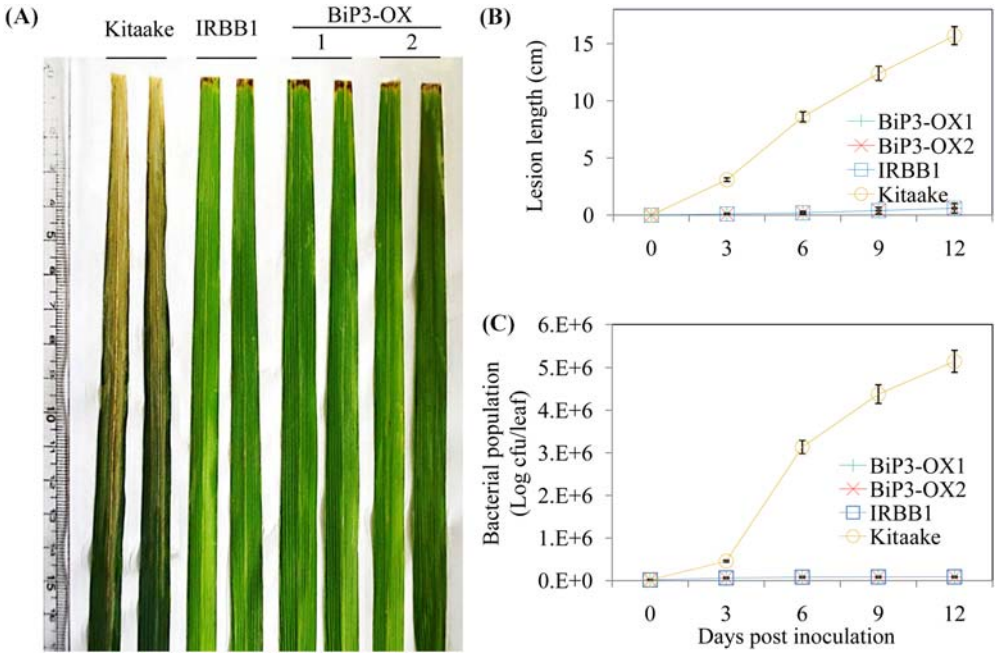


**Fig. 2** Disease phenotype analysis of *BiP3*-OX transgenic plants. (A) Water-soaked lesions on the leaves of Kitaake plants (a susceptible control), IRBB1 (resistant control plants), and BiP3-OX lines. The pictures were taken 12 days after inoculation of these lines with *Xoo* strain KXO85. (B) Leaf lesion lengths of BiP3-OX transgenic and control lines after *Xoo* inoculation of the flag leaves of 10-week-old plants. For each time point, the lesion length was determined separately for three leaves at a similar growth stage from three plants. Each bar represents the average and standard deviation of three leaves. (C) Bacterial population sizes in BiP3-OX transgenic and control lines. For each time point, the bacterial population size was determined separately for three leaves at a similar growth stage from three plants. Error bars represent the standard deviation of three leaves.

folding and maturation of membrane-bound non-RD kinase immune receptors, such as Xa3/Xa26 and Xa21, which pass through the ER during their biogenesis. It is thus likely that the correct folding of the extracellular domain of these immune receptors undergoes quality control in the ER, which would be a critical step in plant immune response pathways. The overexpression of *BiP3* likely perturbs this ER quality control, resulting in incorrect folding of Xa3/Xa26 and Xa21 and impaired resistance to *Xoo*.

In summary, we have here found that the overexpression of *BiP3* in rice does not affect *Xa1*-mediated resistance to *Xoo*. Our results clearly reveal a differential requirement of *BiP3* in rice immune responses to *Xoo* mediated by the non-RD kinases Xa3/Xa26 and Xa21 and by the NB-LRR Xa1 protein product. This indicates that the differential requirement of *BiP3* in rice immunity is not caused by the type of pathogen, but by the type of immune receptor that is involved in the response. Our results provide evidence that *BiP3* regulates the membrane-bound non-RD kinase-mediated immunity but not immune responses mediated by intracellular NB-LRR protein in rice.

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